

# INFLUENZA VACCINES WITH REDUCED AMOUNTS OF SQUALENE

## CROSS REFERENCE TO RELATED APPLICATIONS

This application is a continuation of U.S. application Ser. No. 16/178,897, filed Nov. 2, 2018, now abandoned, which is a continuation of U.S. application Ser. No. 15/007,719, filed Jan. 27, 2016, now U.S. Pat. No. 10,149,901, which is a Continuation of U.S. patent application Ser. No. 13/148,939, filed Dec. 15, 2011, now U.S. Pat. No. 9,278,126, claiming an international filing date of Feb. 10, 2010; which is the National Stage of International Patent Application No. PCT/IB2010/000312, filed Feb. 10, 2010; which claims priority to U.S. Provisional Patent Application No. 61/207,385 filed Feb. 10, 2009; the disclosures of which are herein incorporated by reference in their entirety.

## TECHNICAL FIELD

This invention is in the field of vaccines for protecting against influenza virus infection, and in particular vaccines that include reduced amounts of squalene relative to marketed vaccines.

## BACKGROUND ART

Influenza vaccines generally do not include an adjuvant, except for the PREPANDRIX™ product (GlaxoSmithKline) and the FLUAD™ product (Novartis Vaccines). The adjuvant in the monovalent pre-pandemic seasonal PREPANDRIX™ vaccine is an oil-in-water emulsion. The antigen and the emulsion adjuvant are supplied in separate 10-dose vials for mixing at the point of use at a 1:1 volume ratio. The product datasheet shows that each dose has a volume of 0.5 mL and contains 3.75 µg HA with 10.68 mg squalene and 4.85 mg polysorbate 80.

The adjuvant in the trivalent seasonal FLUAD™ vaccine is an oil-in-water emulsion. The antigen and the emulsion adjuvant are supplied in pre-mixed format in a pre-filled syringe. The product datasheet shows that each dose has a volume of 0.5 mL and contains 15 µg hemagglutinin (HA) per strain with 9.75 mg squalene and 1.175 mg polysorbate 80. As disclosed in reference 1, the vaccine is made by mixing at a 2× emulsion with a 2× antigen solution at a 1:1 volumetric ratio, to give a final solution with both emulsion and antigen at the 1× concentration. This 1:1 mixing ratio is further explained in chapter 10 of reference 75. Modifications of this mixing are disclosed in reference 2.

Compared to the FLUAD™ product, reference 3 discloses trivalent influenza vaccines with lower amounts of HA (3×5 µg HA/dose) and lower amounts of squalene (5.35 mg/dose). The vaccine includes thiomersal preservative and was administered to humans as a 0.5 mL dose.

It is an object of the invention to provide further and improved formulations of adjuvanted influenza vaccines, and in particular of seasonal influenza vaccines.

## DISCLOSURE OF THE INVENTION

In one embodiment, the invention provides an influenza virus vaccine comprising: (i) hemagglutinin from at least one influenza A virus strain and at least one influenza B virus strain, wherein the hemagglutinin concentration is >12 µg/ml per strain; and (ii) an oil-in-water emulsion adjuvant

with submicron oil droplets, comprising squalene, where the squalene concentration is <19 mg/ml.

In another embodiment, the invention provides a mercury-free influenza virus vaccine comprising: (i) hemagglutinin from at least one influenza A virus strain and at least one influenza B virus strain; and (ii) an oil-in-water emulsion adjuvant with submicron oil droplets, comprising squalene, where the squalene concentration is <19 mg/ml.

In another embodiment, the invention provides an influenza virus vaccine having a unit dose volume between 0.2-0.3 mL, wherein the vaccine comprises: (i) hemagglutinin from at least one influenza A virus strain and at least one influenza B virus strain; and (ii) an oil-in-water emulsion adjuvant with submicron oil droplets, comprising squalene, where the squalene concentration is ≤19 mg/ml.

In another embodiment, the invention provides an influenza virus vaccine comprising: (i) hemagglutinin from at least one influenza A virus strain and at least one influenza B virus strain; and (ii) an oil-in-water emulsion adjuvant with submicron oil droplets, comprising squalene, where the squalene concentration is 9.75 mg/mL or 4.88 mg/mL.

In another embodiment, the invention provides an influenza virus vaccine having a unit dose volume between 0.2-0.3 mL, comprising: (i) hemagglutinin from at least one influenza A virus strain and at least one influenza B virus strain; and (ii) an oil-in-water emulsion adjuvant with submicron oil droplets, comprising squalene, where the squalene concentration is 19.5 mg/mL, 9.75 mg/mL or 4.88 mg/mL.

In another embodiment, the invention provides an influenza virus vaccine comprising: (i) hemagglutinin from at least two influenza A virus strains and at least two Influenza B virus strains; and (ii) an oil-in-water emulsion adjuvant with submicron oil droplets, comprising squalene, where the squalene concentration is ≤mg/mL.

## Vaccine Preparation

Various forms of influenza virus vaccine are currently available, and vaccines are generally based either on live virus or on inactivated virus. Inactivated vaccines may be based on whole virions, split virions, or on purified surface antigens. Influenza antigens can also be presented in the form of virosomes. The invention can be used with any of these types of vaccine, but will typically be used with inactivated vaccines.

Where an inactivated virus is used, the vaccine may comprise whole virion, split virion, or purified surface antigens (including hemagglutinin and, usually, also including neuraminidase). Chemical means for inactivating a virus include treatment with an effective amount of one or more of the following agents: detergents, formaldehyde, β-propiolactone, methylene blue, psoralen, carboxyfulerene (C60), binary ethylamine, acetyl ethyleneimine, or combinations thereof. Non-Chemical methods of viral inactivation are known in the art, such as for example UV light or gamma irradiation.

Virions can be harvested from virus-containing fluids by various methods. For example, a purification process may involve zonal centrifugation using a linear sucrose gradient solution that includes detergent to disrupt the virions. Antigens may then be purified, after optional dilution, by diafiltration.

Split virions are obtained by treating purified virions with detergents (e.g. ethyl ether, polysorbate 80, deoxycholate, tri-N-butyl phosphate, Triton X-100, Triton N101, cetyltrimethylammonium bromide, Tergitol NP9, etc.) to produce subvirion preparations, including the 'Tween-ether' splitting process. Methods of splitting influenza viruses are well